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(54) Process for the manufacture of a preparation having a high content of phosphatidylcholine from a phosphatide mixture.

(57) A preparation having a high content of phosphatidylcholine is prepared from a phosphatide mixture by admixing a solution of such a phosphatide mixture in an alcohol having 1–3 carbon atoms with an aqueous or an alcoholic solution of a salt of a bi- or trivalent metal, especially a magnesium salt, and preferably magnesium sulphate, in such an amount that most of the phosphatidylcholine is left in solution whereas most of the other phosphatides are precipitated. The precipitate is separated from the solution and the preparation with a high content of phosphatidylcholine is isolated from the remaining solution by evaporation. The amount of salt to be used is 1–20%, preferably 3–8% by weight relative to the weight of the dry phosphatide mixture.

The starting material can be a crude phosphatide mixture, a desugared and/or defatted mixture, a mixture partly enriched with respect to phosphatidylcholine, etc. Preparations with 70% phosphatidylcholine or higher can be obtained with this process.

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PROCESS FOR THE MANUFACTURE OF A PREPARATION HAVING A HIGH CONTENT OF PHOSPHATIDYLCHOLINE FROM A PHOSPHATIDE MIXTURE

The invention relates to a process for the manufacture of a preparation having a high content of phosphatidylcholine from a phosphatide mixture by admixing an alcoholic solution of the phosphatide mixture with an aqueous or alcoholic solution of a sufficiently soluble salt of a bi- or trivalent metal.

Phosphatides, especially those occurring in crude vegetable fats and oils from which they are isolated by methods known in the art, are esters of phosphatidic acids (diacylglycerylphosphoric acids) and N-containing alcohols such as 2-aminoethanol (colamine), 2-amino-3-hydroxy-propanoic acid (serine), and trimethyl(2-hydroxyethyl)ammonium hydroxide (choline), and N-free hydroxy compounds, such as inositol.

Of these phosphatides, the ester of choline, viz. phosphatidylcholine is rated high as a pharmaceutical compound, as an emulsifier or for dietetic purposes.

Therefore, in both patent and scientific/technological literature processes are described to separate the phosphatides present in phosphatide mixtures and to isolate the fraction wanted. There are processes in which separation is accomplished by using the different solubilities in organic solvents, by adsorption, by chromatographic separation, and by ion-exchange. All these processes have their advantages and their drawbacks.

There are also processes known in which phosphatides are treated with metal salts and isolated as metal salt complexes. As early as 1926 it was described in the Swiss

patent specification No. 127 256 to obtain a lecithin preparation by precipitation from an alcoholic solution with calcium chloride. From the complex obtained the lecithin can be recovered, but from the description it is not clear whether lecithin proper, i.e. phosphatidylcholine, or a phosphatide mixture without much impurity is meant. G. Elenbogen described in the United States patent No. 3 081 320, assigned to Baxter Laboratories Inc., to precipitate an insoluble calcium salt of phosphatidic acids present by adding calciumhydroxide or acetate to a solution of an alcohol-soluble fraction of phosphatide in ethanol; this leaves an alcoholic solution of the phosphatides which are apparently not separated in this way. Other processes are known from German patent No. 1 070 484 to Société Française de Recherches Biochimiques H. Besson & Cie., claiming priority from 27-04-1956 and 29-03-1957. In this specification it is described that a solution of a crude phosphatide mixture, preferably defatted with acetone, in 95-100% ethanol is treated with a solution of magnesium sulphate in methanol or water. When methanol is used, a complex is precipitated. When water is used, a thick paste is obtained which is solidified further by adding acetone. In this process a complex of phosphatidylcholine is precipitated with magnesium sulphate from a lecithin preparation substantially free of phosphatidylcolamine, obtained by treating one part of crude phosphatide mixture with 5-10 parts of magnesium oxide. The precipitate contains up to 28% of magnesium sulphate, which is not removed. The phosphatidylcholine is precipitated by at least 0,5 part by weight of magnesium sulphate heptahydrate per part of phosphatidylcholine.

It has now been found that separation of the phosphatides present in a phosphatide mixture can be accomplished by treating an alcoholic solution of such a mix-

ture with sufficiently soluble salts of di- and trivalent metals dissolved in an alcohol or in water, in an amount which precipitates phosphatidylcolamine and phosphatidylserine (in addition to phosphatidic acid), but substantially leaves phosphatidylcholine in solution.

The following Table shows the effect of this invention (extracted from figures given in the Examples in more detail):

Example	% $MgSO_4$ of amount of starting material	yield(%) of phosphatides	
		phosphatidyl-choline	phosphatidyl-colamine
VI	1.0	100	56
IV	2.4	80	13
I	3.7	79	6
III	3.9	88	12

The phosphatide mixture used as starting material can be the crude mixture as obtained by desliming oils and fats, possibly defatted. One can also start from mixtures which were purified further by removal of sugars, fats and other non-phosphatide contaminants. One can also start from phosphatide mixtures already partly enriched in phosphatidylcholine or fractions remaining from such an enrichment. Although the main purpose may be to obtain preparations with a high proportion of phosphatidylcholine, the fractions which contain high proportions of other phosphatides, such as phosphatidylcolamine need not be discarded but may be used for other useful purposes.

For the present process the phosphatide mixture is dissolved in an aliphatic alcohol with 1-3 carbon atoms;

methanol is preferably used, but ethanol containing up to 25% water, and 2-propanol can also be employed. The concentration is from about 5 wt.% to about 35 wt.%, preferably between 10 and 25 wt.%.

As appears from screening experiments, a lot of water- or alcohol-soluble salts of di- or trivalent cations such as magnesium, zinc, calcium, cadmium and aluminium cations can precipitate phosphatidylcolamine and phosphatidic acid from alcoholic solutions and leave phosphatidylcholine in solution. Obviously, for food and pharmaceutical uses only non-toxic salts are to be used. The salts are, before being admixed with the phosphatide solution, dissolved in a suitable solvent, such as an alcohol or water, in which they must be sufficiently soluble. Both hydrates of the salts and anhydrous salts can be used. Salts that can be used include magnesium sulphate, magnesium chloride, magnesium bromide, magnesium nitrate, and magnesium acetate. Magnesium sulphate is preferred, for both selectivity and toxicological reasons.

The concentration of the salt solution is preferably as high as possible for easy treatment, but lower concentrations can be used without disadvantage. As solvents can be used aliphatic alcohols with 1-3 carbon atoms, methanol being preferred, and water.

The amount of salt, calculated as the anhydrous salt, relative to the weight of the phosphatide starting material is 1-20%, preferably 3-8%, and depends on the content of phosphatides to be precipitated. With higher amounts phosphatidylcholine is also precipitated, with lower amounts phosphatidylcolamine etc. is left in solution.

The temperature at which the precipitation can be performed is 0-75°C, but only slight differences are observed at different temperatures. There is therefore no need to operate at other temperatures than ambient temperature or slightly higher.

After the phosphatide solution and the salt solution has been mixed carefully and the precipitate has been formed, the mixture is stirred well to avoid inclusion of solute in the precipitate as well as possible; the precipitate is then removed from the remaining solution by decanting, by filtration, by centrifuging or by other suitable means. The remaining solution is evaporated, possibly under reduced pressure, leaving a preparation enriched in phosphatidylcholine.

For further enrichment the process can be repeated.

The preparation can further be purified by removing sugars and fats, which can also be done before the precipitation process or repetition of the process.

By the process invented, a preparation containing over 70% or even over 80% phosphatidylcholine can be obtained.

The invention is further illustrated by the following examples, which do not however limit the scope of the invention.

Example I

To a solution of 100 g defatted ethanol-soluble part of soyaphosphatide in one litre methanol, 15 g of an aqueous solution containing 3.7 g magnesium sulphate (concentration 244 g/kg) was added while stirring (amount 0.037 g $MgSO_4$ per g phosphatide). The precipitate was

filtered off using 50 g silicagel as filter aid. The filtered solution was evaporated leaving 65 g product.

The product was analyzed
- for the various phosphatides by thin-layer chromatography according to Fette, Seifen, Anstrichmittel, 63, 1119-1123 (1961);
- for the water-soluble part as follows: 5 g product was dissolved in 200 ml of a mixture of 2 parts of chloroform and 1 part of methanol; 40 ml water were then added and the whole shaken for 2 min. The mixture was centrifuged, the upper phase separated and evaporated in vacuum. The residue, being the water-soluble part, was weighed after drying at 60°C;
- for nitrogen (Kjeldahl);
for magnesium by atomic absorption spectrophotometry (see e.g. W.T. Elwell and J.A.F. Gidley, Atomic-Absorption Spectrophotometry, Oxford et al. 1966, pp. 100-101).

Analysis data:	starting material		product		
	g	%	g	%	yield %
total	100		65		65
phosphatidylcholine	60	60	47.5	73	79
phosphatidylcolamine	11	11	0.7	1	6
lyso-phosphatidyl-choline	3	3	0	0	0
water-soluble part			11.1	17	
nitrogen				1.42	
magnesium				0.44	

Instead of dissolving the defatted ethanol-soluble part of soyaphosphatide in methanol, about the same results are obtained by dissolving the starting material in 1 litre 90% ethanol or in 1 litre 2-propanol.

Example II

To a solution of 100 g of the ethanol-soluble part of soya-phosphatide in 500 g methanol, 16 g of an aqueous solution containing 3.9 g magnesium sulphate (concentration 244 g/kg) was added while stirring (amount 0.039 g $MgSO_4$ per g phosphatide). After removal of the precipitate and evaporation of the remaining solution as in Example I, the residue was first treated with 175 ml of a 1:1 acetone/water mixture and then 4 times with 175 ml acetone. By this aftertreatment sugar and fat are substantially removed.

The product was analyzed as indicated in Example I, and for phosphorus according to method F-I 5a (68), published by Deutsche Gesellschaft für Fettwissenschaft, Deutsche Einheitsmethoden (Stuttgart 1950-1975), and for fat/oil according to H. Pardun, Fette, Seifen, Anstrichmittel 66, 467 (1964).

Analysis data:	starting material			product	
	g	%	g	%	yield (%)
total	100		35		35
phosphatidylcholine	32	32	27.7	79	86
phosphatidylcolamine	6	6	0.4	1	6.7
lyso-phosphatydyl-choline	11	11	2.7	4.8	24.8
other phosphatides				3	
nitrogen				1.54	
phosphorus		2		3.44	
magnesium				0.7	
fat/oil	40	40	0.2	0.6	0.5
water-soluble part	8	8		6.7	29.3

Example III

100 g of an alcohol soluble fraction of desugarized soyaphosphatide was dissolved in 400 ml methanol. To this solution 16 g of an aqueous solution containing 3.9 g $MgSO_4$ was added dropwise, while stirring. After the addition was completed, stirring was continued for another 60 min at room temperature; the precipitate was then removed by centrifuging, and washed with 50 ml methanol. The combined methanolic solution was evaporated. The dry residue (70 g) was deoiled by treatment with methyl acetate, leaving 57 g product.

The product was analyzed as indicated in the previous Examples.

Analysis data:	starting material		product		
	g	%	g	%	yield (%)
total	100		57		57
phosphatidylcholine	52	52	46	80	88
phosphatidylcolamine	10	10	1.1	2.0	12
lyso-phosphatidyl-choline	<1	<1		1	
phosphorus		2.9		3.6	
oil/fat	15	15			

Example IV

22.3 kg of an alcohol soluble fraction of desugarized soyaphosphatide was dissolved in 112 kg methanol. To this solution 2.16 kg of an aqueous solution containing 0.53 kg $MgSO_4$ was added at room temperature, while stirring. After 0.5 h stirring the precipitate was allowed to settle overnight, whereupon the supernatant was removed by decantation. After evaporation of the solution obtained 14.8 kg (66%) of a solid product was obtained. The product was analyzed as indicated in the first two Examples.

Analysis data:	starting material		product		
	g	%	g	%	yield (%)
total	22300		14800		66
phosphatidylcholine	11150	50	8880	60	80
phosphatidylcolamine	2230	10	296	2	13
lyso-phosphatidyl-choline	669	3	592	4	
phosphorus		2.7		2.9	
magnesium				0.4	
oil	4906	22	2220	15	45

Example V

100 g soyaphosphatide were extracted with 500 ml methanol at 50°C stirring for 1 h. The mixture was allowed to cool to ambient temperature and the clear supernatant was decanted. To the clear solution obtained, 6.2 g of an aqueous solution containing 1.5 g $MgSO_4$ (concentration 244 g/kg) were added while stirring. The precipitate (12.3 g) was removed and the solution obtained was evaporated. The residue was washed with a mixture of 50 ml hexane (petrol-ether), 50 ml methanol and 50 ml water. The two layers were separated by centrifuging and the aqeous methanol layer washed with 25 ml hexane. The combined hexane layers were evaporated and the residue was defatted by dissolving 4 times in 30-40 ml methyl acetate at 40°C and precipitating by chilling at 0°C. Yield: 12 g product.

Analysis data:	g	%
total	12	
phosphatidylcholine	7.9	66
phosphatidylcolamine	0.3	2.7
lyso-phosphatidylcholine	0.2	1.6
nitrogen		1.55
phosphorus		3.27

Example VI

To a solution of 100 g defatted ethanol-soluble part of soyaphosphatide in 400 ml methanol, 4 g of an aqueous solution containing 1 g magnesium sulfate (concentration 244 g/kg) was added while stirring. The precipitate was allowed to settle overnight, and the clear supernatant was isolated by decanting. After evaporation 77 g product was obtained.

Analysis data:	starting material		product		
	g	%	g	%	yield (%)
total	100		77		77
phosphatidylcholine	32.5	32.5	32.4	42.1	100
phosphatidylcolamine	6.2	6.2	3.4	4.5	56

Example VII

14.8 g desugarized ethanol-soluble part of soyaphosphatide were dissolved in 98 ml methanol. To this solution 10 ml of a freshly prepared solution containing 1.46 g magnesium sulphate heptahydrate in methanol were added dropwise while stirring. After 15 min the precipitate was allowed to settle, and subsequently the supernatant was separated therefrom by decanting. The supernatant left on evaporation 11.0 g of a solid product.

Analysis data:	starting material		product		
	g	%	g	%	yield (%)
total	14.8		11.0		74
phosphatidylcholine	7.4	50	5.6	51	76
phosphatidylcolamine	1.5	10	0.1	1	7

CLAIMS

1. Process for the manufacture of a preparation having a high content of phosphatidylcholine from a phosphatide mixture by admixing a solution of the phosphatide mixture in an alcohol containing 1-3 carbon atoms with an aqueous or alcoholic solution of a sufficiently soluble salt of a bi- or trivalent metal, and separating the precipitate formed and the remaining solution, characterized in that the di- or trivalent metal salts containing solution is admixed in an amount which leaves most of the phosphatidylcholine in solution and precipitates most of the other phosphatide compounds present in the phosphatide mixture, and the solvent of the solution remaining after separation of the precipitate is evaporated.

2. Process according to claim 1, characterized in that a salt of magnesium is used.

3. Process according to claim 2, characterized in that magnesium sulphate is used.

4. Process according to claims 1 - 3, characterized in that 1 - 20% by weight of the salt relative to the weight of the dry phosphatide mixture is used for the precipitation.

5. Process according to claim 4, characterized in that 3 - 8% by weight of the salt relative to the weight of phosphatide is used.

6. Process according to claims 1 - 5, characterized in that an aqueous solution of the metal salt is used.

7. Process according to claims 1 - 6, characterized in that the phosphatide mixture is dissolved in methanol or ethanol or a mixture thereof.

European Patent Office

EUROPEAN SEARCH REPORT

0090454
Application number

EP 83 20 0390

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
A	EP-A-0 043 018 (NATTERMANN)	1	A 23 J 7/02 C 07 F 9/10
A	GB-A- 529 114 (M. SATO et al.)	1	
A	Patent Abstracts of Japan vol. 4, no. 124, 2 September 1980 page 28C23 & JP-A-55-76892	1	
A	Patent Abstracts of Japan vol. 4, no. 93, 5 July 1980 page 5C17 & JP-A-55-54861	1	
A	Patent Abstracts of Japan vol. 3, no. 19, 17 February 1979 page 1C37 & JP-A-53-141300	1	
A,D	DE-C-1 070 484 (STE FRANCAISE DE RECHERCHES BIOCHIMIQUES H. BESSON & CIE) * Column 2, lines 37-42 *		

TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

A 23 J 7/00
A 23 J 7/02
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	16-05-1983	KAPTEYN H G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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